STIC-ILL QP501, J6

From: Ramirez, Delia
Sent: Friday, July 25, 2003 7:21 PM
To: STIC-ILL
Subject: case 09/606129

Hi,

I would like to request photocopies of the following material:

Saito N, Shirai Y.
Protein kinase C gamma (PKC gamma): function of neuron specific isotype.
J Biochem (Tokyo). 2002 Nov;132(5):683-7. Review.

Kawakami T, Kawakami Y, Kitaura J.
Protein kinase C beta (PKC beta): normal functions and diseases.
J Biochem (Tokyo). 2002 Nov;132(5):677-82. Review.

Nakashima S.
Protein kinase C alpha (PKC alpha): regulation and biological function.
J Biochem (Tokyo). 2002 Nov;132(5):669-75. Review.

Shirai Y, Saito N.
Activation mechanisms of protein kinase C: maturation, catalytic activation, and targeting.
J Biochem (Tokyo). 2002 Nov;132(5):663-8. Review.

Thank you,

Delia M. Ramirez, Ph.D.
Patent Examiner
Recombinant Enzymes-Art Unit 1652
USPTO
1911 S. Clark Street, Crystal Mall 1, 10D06, Mail room 10D01
Arlington, VA 22202
(703) 306-0288
delia.ramirez@uspto.gov

 J. Biochem. 132, 669–675 (2002)

Protein Kinase Cα (PKCα): Regulation and Biological Function

Shigeru Nakashima[1]

Department of Biochemistry, Gifu University School of Medicine, Tsukasamachi 40, Gifu 500-8705

Received July 12, 2002; accepted September 25, 2002

Protein kinase Cα (PKCα) is a serine/threonine kinase and a member of the conventional (classical) PKCs (cPKCs), which have four conserved (C1 to C4) regions. This ubiquitously expressed PKC isotype is activated in response to many different kinds of stimuli and translocates from cytosol to the specialized cellular compartments (nucleus, focal adhesion, caveolae, *etc.*) where it is presumed to work. Therefore, PKCα has been implicated in a variety of cellular functions including proliferation, apoptosis, differentiation, motility, and inflammation. However, the responses induced by activation or overexpression of PKCα vary depending on the types, and sometimes conditions, of cells. For example, in some types of cells, PKCα is implicated in cell growth. In contrast, it may play a role in cell cycle arrest and differentiation in other types of cells. Therefore, alterations of cell responses induced by PKCα are not an intrinsic property of this isoform. The responses are modulated by dynamic interactions with cell-type specific factors: substrates, modulators and anchoring proteins.

Key words: apoptosis, differentiation, migration, proliferation, translocation.

1. Introduction

Mammalian PKCα consists of 672 amino acids and is distributed in all tissues, in contrast to other PKC isotypes whose expression is restricted in the particular tissues (*1, 2*). PKCα is activated by a variety of stimuli, including signals binding to guanine-nucleotide-binding protein-coupled receptors and to tyrosine kinase receptors (*1–5*), and also physical stresses like hypoxia (*6*) and mechanical strain (*7*). Therefore, this isotype plays important roles in the control of major cellular functions: proliferation, apoptosis, differentiation, motility and so on. However, the distinct cellular responses are not due to a multipotential property of this isotype but are regulated through its dynamic interactions with special factors. In other words, the output after activation absolutely depends on where and when it is activated, and what substrates it acts on. However, no complete list of these cell-specific factors is yet available. This review discusses the current understanding of activation mechanisms and possible biological functions of PKCα.

2. Activation and translocation

PKCα is activated by a variety of stimuli originating from receptor activation, cell contact and physical stresses. Diacylglycerol (DG) and Ca^{2+} increased in the cell upon stimulation synergistically drive the release of a pseudosubstrate region from the active site, leading to activation (*8*). Also, kinase activity of PKCα is regulated by phosphorylation of three conserved residues in its kinase domain: the activation-loop site Thr-497, the autophosphorylation site Thr-638, and the hydrophobic C-terminal site Ser-657 (*9, 10*). PKCα exhibits almost no activity without phosphorylation at these sites. Phosphorylation of Ser-657 is currently used as a marker for PKCα activation (*7, 9*). The general mechanisms of PKC activation are discussed in the other article in this minireview series. Here, the possible regulatory mechanisms for stimulus-induced translocation to the particular cellular compartments and signal relays between other important signaling pathways are briefly introduced.

2.1. Translocation. Upon stimulation, PKCα translocates from cytosol to a so-called particulate fraction. This movement was first identified by Western blotting using a specific antibody against PKCα after fractionation of cells and later by immunofluorescent study. Recently, new approaches to analyze the spatial distribution of the protein in cells, such as fluorescence resonance energy transfer (FRET) and confocal microscopy, provide a method for identifying the exact location of PKCα. The application of the fusion protein consisting of PKCα and jellyfish green fluorescent protein (GFP) allows resolution of its distribution in living cells (*11*). These new approaches (*11–13*) revealed that PKCα redistributed from cytosol to the entire membrane in response to non-specific activator for PKCs such as phorbol myristate acetate (PMA). However, the specific stimuli cause translocation of PKCα to the plasma membrane, where DG is presumed to be produced, and also to the specialized cell compartments such as nuclei, focal adhesions, regions of cell–cell contact and so on.

One of the compartments is the nucleus (*14*). In fibroblasts, growth factors like PDGF (platelet-derived growth factor), EGF (epidermal growth factor), and IGF-1 (insulin-like growth factor–1) cause redistribution of PKCα to the nucleus. PKCα, like other PKC isotypes, does not contain the nuclear localization signals (NLS). Although the exact mechanism of nuclear translocation is not yet known, the components for NLS-dependent transport, importin and Ran/GTPase, are not necessary (*15*).

Focal adhesions and regions of cell–cell contact are other common compartments (*13, 16, 17*). Accumulation of PKCα

[1]For correspondence. Tel/Fax: +81-58-267-2228, E-mail: bio@cc.gifu-u.ac.jp

in focal adhesions and lamellipodia is often observed when cells spread and migrate. Upon wounding, epithelial cells migrate from the edge to reepithelialize the wound. In the scratch wounding model of cultured Madin-Draby canine kidney (MDCK) epithelial cells, PKCα translocates to the cell periphery at the wound edge and becomes concentrated in lamellipodia during migration (*18*).

2.2. Anchoring proteins. The proteins which interact with specific PKC isoforms for localization at the specialized cell compartments are called anchoring (or docking) proteins (*19*). In the absence of these isoform-specific (and probably tissue-specific) anchoring proteins, translocation may not be strictly defined and may depend on variations of DG and Ca^{2+} concentrations. RACK1 (receptor for activated protein kinase C 1), which was the first anchoring protein to be isolated for PKCβII, was shown to bind several PKC isoforms including PKCε and PKCα (*20*). The proteins responsible for localization of PKCα at focal contacts have been identified by overlay assay and density gradient cell fractionation. Vinculin and talin, two focal contact proteins were isolated by overlay assay in REF52 cells (*21*). β1–integrin co-sedimented with PKCα in velocity sucrose gradient centrifugation in MCF-7 cells (*13*). Caveolin was identified as an anchoring protein that recruits PKCα to caveolae (*22*). However, the proteins implicated in localization of PKCα at the nucleus are still unknown.

2.3. Signal relay. Upon activation, PKCα is presumed to phosphorylate a variety of substrates depending on the types of stimuli and cells. The identification of substrates in distinct cellular responses will provide a clue to understanding the roles of PKCα in biological functions. However, no complete list of substrates is available. Here, the important signaling pathways that interact with PKCα are introduced (Fig. 1).

The extracellular signal–regulated kinase/mitogen-activated protein kinase (ERK/MAPK) cascade, which involves the kinases Raf, MAPK/ERK kinase (MEK), and ERK/MAPK, is ubiquitously expressed and serves to alter cell functions. The activated ERK finally moves into the nucleus, where it phosphorylates target molecules such as transcription factors, Elk-1 and SAP1. PKCα functions as a potent activator of c-Raf-1 and turns on the ERK/MAPK cascade (*23, 24*).

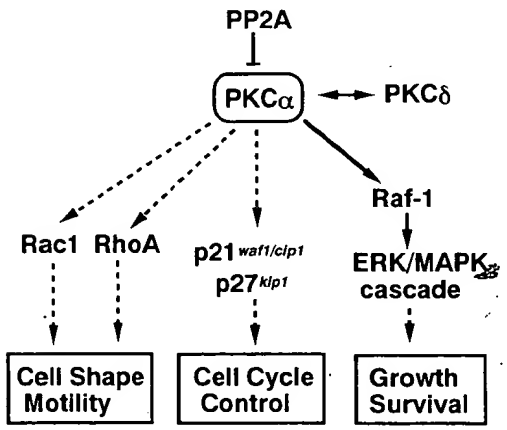

Fig. 1. **Possible interactions of PKCα with other signaling pathways.** →, signal transduction pathways, direct (solid lines), and indirect (broken lines); ⊣, inhibition; ↔ antagonistic regulation.

The low molecular weight GTP-binding proteins of the Rho family, Rho, Rac, and cdc42, have been assigned pivotal roles in actin stress fiber formation, membrane ruffling, cell movement and transcription factor activation. Rac1 acts downtream of PKCα in lamellipodia formation and migration (*25*). Moreover, PKCα is shown to closely interact with RhoA in transcription factor AP-1 activation in T cells (*26*) and in caveolae formation in carbachol-stimulated smooth muscle cells (*27*).

PKCα is implicated in cell growth and differentiation, and thus control of cell cycle machinery. Although the exact signaling cascade is not known, PKCα has been shown to induce cyclin-dependent kinase inhibitors, $p21^{Waf1/Cip1}$ and $p27^{Kip1}$ in several types of cells (*28–30*).

Several biological responses, such as proliferation and apoptosis, are inversely regulated by PKCα and PKCδ (*2, 31–34*). It appears that these two isotypes mutually regulate the expression and activity of other isoforms (*31, 32*).

As mentioned above, PKCα kinase activity is regulated by phosphorylation. Since PKCα activity can be inhibited through its dephosphorylation by protein phosphatase 2A (PP2A), an okadic acid–sensitive phosphatase, the termination of PKCα activity is assumed to be partly mediated by PP2A (*5*). During activation and translocation of PKCα by PMA, PP2A also translocates to the membrane and is coimmunoprecipitated with PKCα (*35*). Therefore, it is likely that PP2A is physically associated with PKCα and plays a role in the termination of its activation through dephosphorylation.

3. Biological functions

PKCα is implicated in a variety of biological responses. Selective activators and inhibitors of PKC, and downregulation by the prolonged incubation of cells with phorbol esters, have provided useful information to analyze the roles of PKCs in biological functions. However, such approaches do not discriminate the specific roles of individual isoforms. In PKC isoforms that are expressed only in particular tissues, such as PKCγ, PKCδ, PKCε, PKCζ, and PKCθ, functional analysis is performed in the null mice by homologous recombination (this will be discussed in other articles in this minireview series). However, PKCα is expressed ubiquitously in tissues and is implicated in oocyte development and fertilization (*36*). The implication of PKCα in specific cell responses is analyzed in culture cell systems by introduction of wild-type PKCα or its dominant negative (kinase-dead) mutant *via* transfection or adenoviral infection. Specific targeting of PKCα by antisense oligonucleotides or by ribozymes also provides similar specificity for *in vitro* and *in situ* studies. The biological responses obtained by the manipulation of PKCα are cell-type specific. For example, overexpression of PKCα promoted proliferation in some types of cells, but caused cell cycle arrest and differentiation in other types. These observations indicate that alterations of cell responses induced by PKCα overexpression are not an intrinsic property of this isoform. The responses are modulated by dynamic interactions with cell-type specific factors: substrates, modulators, and anchoring proteins.

3.1. Proliferation. The implication of PKC in cell growth was first demonstrated in quiescent Swiss 3T3 cells stimulated by PMA. Several growth factors, such as PDGF,

activate and cause nuclear translocation of PKC. Transfection studies indicate that overexpression of PKCα is sufficient to promote proliferation in some types of cells. For example, overexpression of PKCα in the human glioma U87 cell line resulted in enhanced cell proliferation and decreased expression of glial fibrillary acidic protein (GFAP), a glial diffentiation marker (*34*). Overexpression of PKCα in MCF-7 human breast cancer cells leads to a more aggressive phenotype, and cells exhibit an enhanced proliferation rate, anchorage-independent growth in soft agar, and increased tumorigenisity in nude mice (*37*). The proliferative effect of PKCα appears to be mediated via activation of the extracellular signal-regulated kinase/mitogen-activated protein kinase (ERK/MAPK) cascade initiated by Raf-1 (*23, 24*), and/or upregulation of $p21^{waf1/Cip1}$ (*28*), which facilitate formation of complex between cyclin and cyclin-dependent kinase (CDK).

In contrast, overexpression of PKCα results in cell cycle arrest and growth inhibition in several types of cells. In contrast to MCF-7 cells, transfection of PKCα in MCF-10 human mammary epithelial cell line resulted in slower growth with extended doubling time compared to parent cells (*25*). Murine fibroblast cell lines R6, BALB/C, and Ψ2 expressing PKCα grew slower, as assessed by growth rate and saturation densities, than their parent cells (*38*). Another study (*39*) confirmed the inhibition of proliferation by PKCα in R6 cells, although overexpression of PKCα enhanced PMA-induced expression of growth-regulatory genes c-*jun*, c-*myc*, and collagenase. In the IEC-18 intestinal crypt cell line (*30*), PKCα activation appeared to lead to cell cycle arrest by accumulation of the hypophosphorylated growth-suppressive form of the retinoblastoma protein (Rb) and induction of the cyclin-dependent kinase inhibitors, $p21^{Waf1/Cip1}$ and $p27^{Kip1}$. Therefore, PKCα is closely involved in the regulation of the cell cycle.

3.2. Apoptosis. Apoptosis is a genetically programmed form of cell death, which is important in morphogenesis and development, and for the removal of damaged cells as well as tumor cells. The role of PKCα in apoptosis also depends on cell types (Fig. 2).

PKCα exhibits an anti-apoptotic function and may function as a survival factor in some types of cells (*2*). This notion is based on the observations that PKCα is often blocked during apoptotic processes. In some cases PKCα undergoes proteolytic hydrolysis by calpain and/or caspases. Ceramide, a known inducer of apoptosis, causes inactivation of PKCα, probably through dephosphorylation by phosphatases (*40*). During induction of apoptosis by Fas ligation in Jurkat human lymphoblstoid T cells (*41*), the activity of PKCα is inhibited through activation of okadaic acid-sensitive phosphatase, PP2A. Induction of apoptosis of Jurkat cells by α-tocophenyl succinate, a vitamin E analog, is accompanied by the inhibition of PKCα through increased activity of PP2A (*42*). Upregulation and downregulation of PKCα also confirmed its anti-apoptotic role. Cells transfected with wild-type PKCα became resistant to apoptosis (*43*). In contrast, inhibition of PKCα by antisense oligonucleotides increased the sensitivity to apoptotic inducers (*42, 43*). In COS-1 cells, expression of dominant negative PKCα is sufficient to induce apoptosis (*44*). Jurkat cell line was sensitive to apoptosis induced by α-tocophenyl succinate. Jurkat cells overexpressing PKCα became less susceptible to α-tocophenyl succinate–induced apoptosis (*42*). In contrast, antisense oligonucleotide to PKCα increased apoptosis after exposure to α-tocophenyl succinate. In glioblastoma cells (*45*), down-regulation of PKCα by antisense oligonucleotide was accompanied by the induction of p53 prior to the appearance of apoptotic cells. p53 is known to regulate the expression of proapoptotic proteins, Bax, Apaf-1, caspases, and insulin-like growth factor binding protein–3 (IGFBP-3). Expression of IGFBP-3 induced apoptosis of glioma and breast cancer cells. These observations suggest that PKCα protects cells from apoptosis, in part through blockage of p53 induction. The anti-apoptotic action of PKCα is also shown to be mediated via activation of the Raf-1/ERK/MAPK cascade (*23, 24*) and/or phosphorylation of anti-apoptotic protein Bcl2 (*46*).

On the other hand, PKCα may also play a proapoptotic role. PMA promoted death of human gastric cancer cell lines MKN45 and MKN47 with increase of activity of PKCα, but not PKCβ, when they lost anchorage (*47*). Overexpression of PKCα *via* vaccinia viruses augmented apoptosis of detached MKN45 and MKN47 cells, leaving the viability of the attached cells unaffected. These observations indicate that integrin-mediated signals inhibit the apoptotic action of PKCα. PKCα may play a role in the induction of apoptosis of p53-null HL60 human leukemia cells induced by camptothecin, a DNA topoisomerase I

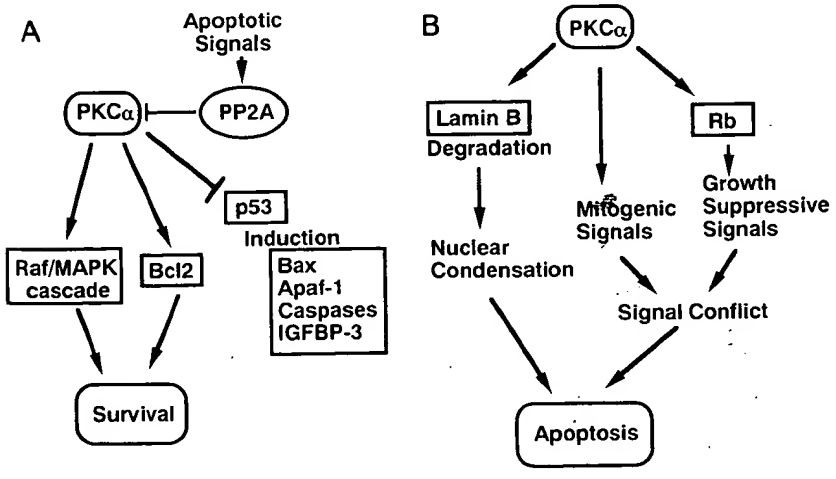

Fig. 2. **Possible involvement of PKCα in both antiapoptotic (A) and proapoptotic (B) signaling.**

inhibitor (*48*). PKCα is implicated in phosphorylation of nuclear lamin B, which promotes disassembly of nuclear lamina. Therefore, PKCα-mediated phosphorylation and subsequent solubilization (degradation) of lamin B is likely to affect nuclear and chromatin structure. The proapoptotic role of PKCα in LNCaP human prostate cancer cells is complicated (*49, 50*). Apoptosis of this cell line by PMA is initiated by a conflict between growth-suppressive signals from RB and growth-promoting mitogenic signals. The involvement of PKCα in both growth-suppressive and mitogenic signals is confirmed by its inducible expression. This signal conflict was blocked by cell aggregation through E-cadherin–mediated signals and the aggregated cells survived.

3.3. Differentiation. Cell differentiation is accompanied by the inhibition of cell cycle progression and expression of cell-specific functions. In some types of cells, such as hematopoietic progenitor cells (*51, 52*), lens epithelial cells (*53*), F9 embryonal carcinoma cells (*54*), and melanoma cells (*55*), PKCα is closely involved in differentiation. In the development of hematopoietic cells, the progression of erythroid progenitor cells (*51*) and development of macrophages are controlled by PKCα (*52*). Stimulation of hematopoietic granulocyte macrophage colony forming cells (GM-CFC) with macrophage colony stimulating factor (M-CSF) leads to macrophage formation with translocation of PKCα to the nucleus. When GM-CFC are transfected with a constitutively activated form of PKCα that is devoid of the N-terminal regulatory domain, the expressed protein is located primarily in the nucleus. These transfected cells are committed to development toward macrophage lineage even in the presence of factors that normally promote only neutrophilic development. Overexpression of PKCα in melanoma cells resulted in elongation of doubling time, diminished anchorage-independent growth in soft agar, and increased melanin production (*56*). PKCα also takes part in the chondrogenesis of mesenchymal cells (*57*). The details of molecular mechanisms of PKCα-mediated differentiation remain to be elucidated. Translocation to the nucleus during differentiation suggests the possibility that PKCα may be implicated in cell cycle control and/or expression of genes necessary for the differentiation phenotype.

The possible involvement of PKCα in oocyte maturation and morphogenesis has also been demonstrated. In porcine oocytes (*36*), injection of an antibody specific for PKCα blocked cortical granule exocytosis by PMA or fertilization. F9 embryonal carcinoma cells differentiate into parietal endoderm-like cells in response to retinoic acid (RA) (*54*). Undifferentiated cells express PKCβ but not PKCα, whereas differentiated endoderm cells express PKCα but not PKCβ. Overexpression of PKCα in F9 cells enhanced RA-induced differentiation, indicating an important role of PKCα in induction and maintenance of endoderm phenotype.

3.4. Cell migration and adhesion. As PKCα translocates and accumulates in focal contacts, it is shown to be involved in the regulation of cell motility and the adhesion of focal contacts. In MDCK cells (*18*), PKCα is the only PKC isoform that translocates and participates in the formation of Ca^{2+}-dependent desmosomes in response to scratch wounding of a confluent cell sheet. An antisense oligonucleotide to PKCα abolishes the development of desmosomes at the wound edge. The role of PKCα in wounding closure has also been demonstrated in MCF-10A and 2C4 fibrosarcoma cells (*13*). At the wound edge, PKCα interacts with β1-integrin and ERZ (ezrin, radixin, and moesin) proteins, F-actin binding proteins which are involved in the maintenance of cell shape and extension of lamellipodia. Stable expression of PKCα increased phosphorylation of ERZ proteins at C-terminal threonine. Overexpression of ezrin mutant at the C-terminal phosphorylation site inhibited PKCα-mediated cell migration, indicating that migration is controlled through the phosphorylation of ERZ proteins by PKCα. Implication of small G protein Rac1 in PKCα-mediated cell migration has been demonstrated in MCF-10 cells (*25*). Overexpression of PKCα in MCF-10 cells enhanced cell motility. This increased motility is blocked by PKC inhibitors and dominant-negative Rac1, but not by dominant-negative RhoA or dominant-negative Cdc42. As mentioned below, PKCα participates in migration of vascular endothelial and smooth muscle cells.

3.5. Tumorigenesis. The malignant phenotype of carcinoma cells is associated with uncontrolled growth, morphological change and invasion. Therefore, PKCα is implicated in malignant phenotypes of several tumors, such as gliomas and breast cancers. In fact, elevated PKC activity was observed in human breast tumors. Moreover, overexpression of PKCα caused human breast cancer cells to show a more aggressive and metastatic phenotype, anchorage-independent growth in soft-agar and tumorgenicity in nude mice (*25, 37*). PKCα may also be involved in the drug resistance of cancer cells through phosphorylation and activation of a membrane-bound efflux pump, termed P-glycoprotein, the product of mdr1 gene. An MCF-7 cell colony isolated on the basis of its resistance to doxorubicin expressed elevated levels of PKCα (*37*). On the other hand, in doxorubicin-resistant MCF-7 cells transfected with PKCα, the resistance to anti-cancer drugs was increased with enhanced phosphorylation of P-glycoprotein and decreased drug accumulation (*58*).

A mutant form of PKCα (four point mutations in the regulatory domain) was isolated from a murine fibrosarcoma cell line by ultraviolet irradiation and was reported to have the ability to transform Balb/c fibroblasts (*59*). However, the tumorgenic activity of this mutant PKCα could not be confirmed by the other investigators (*38*). A point mutation in the V3 region (D294G) of PKCα was identified in human pituitary and thyroid tumors (*60*). Ectopic expression of D294G PKCα in rat embryonic fibroblasts displayed a decreased requirement of serum for proliferation, and these cells formed colonies in soft agar (*61*). However, this mutant failed to transform human pituitary cell line GH3B6 (*60*). These observations indicate that the function of PKCα is strictly regulated by intracellular conditions.

If unregulated growth and invasiveness are, in part, regulated by PKCα, it could be a target for anti-cancer therapy. In fact, antisense blocking of PKCα reversed the transformed phenotype of human lung carcinoma cells (62) and glioma cells (*63*). All-*trans*-retinoic acid (ATRA), a vitamin A derivative, has the ability to reverse the malignant to the normal phenotype and to inhibit cancer invasiveness and unregulated growth, and is used to treat several types of cancer. PKCα appears to be a possible target of ATRA (*64*). ATRA is shown to bind to PKCα through competition with PS and inhibit its activity.

3.6. Cardiac hypertrophy and agiogenesis. PKCα

regulates the hypertrophic growth of neonatal cardiomyocytes, characterized by enhanced sarcomeric organization, increased cell surface area, increased expression of atrial natriuretic factor, and increased [^{3}H]leucine incorporation. PKCα antisense oligonucleotides inhibited hypertrophic growth of rat neonatal cardiomyocytes induced by myotrophin (*65*). In contrast, overexpression of wild-type PKCα induced hypertrophic growth of neonatal cardiomyocytes (*66*). Dominant negative PKCα antagonized phenyrephrine-induced hypertrophic growth and PMA-induced activation of ERK1/2. On the other hand, dominant negative MEK1, an upstream regulator of ERK1/2, inhibited hypertrophy induced by wild-type PKCα. Therefore, PKCα mediates hypertrophic growth of neonatal cardiomyocytes, in part through activation of the ERK1/2-dependent signaling pathway.

Overexpression of PKCα in rat capillary endothelial cells enhanced their migration in response to hepatocyte growth factor (HGF), known as "scatter factor" (*67*). Antisense study indicates that PKCα is required for migration of human umbilical endothelial cells in response to scratch-wounding (*68*). PKCα is also implicated in migration of vascular smooth muscle cells, since its down-regulation by antisense oligonucleotides resulted in the inhibition of cell spreading toward fibronectin (*16*). These findings suggest that PKCα plays an important part in vascular formation.

3.7. Inflammation. The possible implication of PKCα in inflammatory responses has also been suggested. Dominant negative PKCα inhibited bacterial lipopolysaccharide-induced cytokine production by macrophages (*61*). Production of nitric oxide (NO), an inflammatory mediator induced by lipopolysaccharides in vascular smooth muscle cells, is enhanced by overexpression of PKCα (*70*). Overexpression of PKCα in the epidermis of transgenic mice by keratin 5 promoter resulted in striking alterations of PMA-induced inflammatory responses, edema and infiltration of neutrophils, and expression of genes implicated in inflammation such as cyclooxygenase-2 and tumor necrosis factor–α (*71*). Overexpression of PKCα in human keratinocytes had no effect on proliferation or differentiation (*72*). Consistent with this observation, the sensitivity to PMA-induced tumor promotion in the skin of transgenic mice was identical to that of wild-type mice. Inconsistent with these observations, PKCα appeared to inhibit secretory responses and release of arachidonic acid (AA) metabolites in the mast cell line RBL-2H3 cells (*73, 74*). The possible involvement of PKCα in the inhibition of inflammatory responses is also suggested in HepG2 human hepatocellular cell line stimulated by interlukin-1 (IL-1). Down-regulation of PKCα by antisense oligonucleotides blocked IL-1–induced expression of IκB (inhibitor of NFκB) (*75*). Therefore, PKCα negatively regulates NFκB-induced expression of genes involved in a variety of inflammatory responses through induction of IκB.

4. Conclusion

PKCα was first thought to be activated in a Ca^{2+}–mobilizing signal transduction system. Extensive studies in the past two decades have revealed that it functions in a variety of cell responses including one which is initiated by the tyrosine kinase, and that its regulatory mechanisms are more complicated than originally imagined. It is now clear that localization of PKCα at particular cell compartments is essential for it to exhibit specific functions. Further exploration of the specific substrates and anchoring proteins will lead to the better understanding of the networks of PKCα signal transduction pathways.

REFERENCES

1. Nishizuka, Y. (1992) intracellular signaling by hydrolysis of phospholipids and activation of protein kinase C. *Science* **258**, 607–614
2. Dempsey, E.C., Newton, A.C., Mochly-Rosen, D., Fields, A.P., Reyland, M.E., Insel, P.A., and Messing, R.O. (2000) Protein kinase C isozymes and the regulation of diverse cell responses. *Am. J. Physiol.* **279**, L429–L438
3. Newton, A.C. (1995) Protein kinase C: Structure, function, and regulation. *J. Biol. Chem.* **270**, 28495–28498
4. Nishizuka, Y. (1995) Protein kinase C and lipid signaling for sustained cellular responses. *FASEB J.* **9**, 484–496
5. Parekh, D.B., Ziegler, W., and Parker, P.J. (2000) Multiple pathways control protein kinase C phosphorylation. *EMBO J.* **19**, 496–503
6. Lo, L-W., Cheng, J-J., Ciu, J-J., Wung, B-S., Liu, Y-C., and Wang, D.L. (2001) Endothelial exposure to hypoxia induces Rge-1 expression involveing PKCα-mediated Ras/Raf-1/ERK1/2 pathway. *J. Cell. Physiol.*, **188**, 304–312
7. Cheng, J-J., Wung, B-S., Chao, Y-J., and Wang, D.L. (2001) Sequential activation of protein kinase C (PKC)-α and PKC-ε contributes to sustained Raf/ERK1/2 activation in endothelial cells under mechanical strain. *J. Biol. Chem.*, **276**, 31368–31375
8. Dutil, E.M. and Newton, A.C. (2000) Dual role of pseudosubstrate in the coordinated regulation of protein kinase C by phosphorylation and diacylglycerol. *J. Biol. Chem.* **275**, 10697–10701
9. Bornancin, F. and Parker, P.J. (1997) Phosphorylation of protein kinase C-α on serine 657 controls the accumulation of active enzyme and contributes to its phosphatase-resistant state. *J. Biol. Chem.* **272**, 3544–3549
10. Hansra, G., Garcia-Paramio, P., Prevostel, C., Whelan, R.D.H., Bornancin, F., and Parker, P.J. (1999) Multiple dephosphorylation and desensitization of conventional protein kinase C isotypes. *Biochem. J.* **342**, 337–344
11. Wagner, S., Harteneck, C., Hucho, F., and Buchner, K. (2000) Analysis of the subcellular distribution of protein kinase Cα using PKC-GFP fusion proteins. *Exp. Cell Res.* **258**, 204–214
12. Ng, T., Squire, A., Hansra, G., Bornancin, F., Prevostel, C., Hanby, A., Harris, W., Barnes, D., Schmidt, S., Mellor, H., Bastiaens, P.I.H., and Parker, P.J. (1999) Imaging protein kinase Cα activation in cells. *Science*, **283**, 2085–2089
13. Ng, T., Parsons, M., Hughes, W.E., Monypenny, J., Zicha, D., Gautreau, A., Arpin, M., Gschmeissner, S., Verveer, P.J., Bastiaens, P.I.H., and Parker, P.J. (2001) Ezrin is a downstream effector of trafficking PKC-integrin complexes involved in the control of cell motility. *EMBO J.* **20**, 2723–2741
14. Buchner, K. (1995) Protein kinase C in the transduction of signals toward and within the cell nucleus. *Eur. J. Biochem.* **228**, 211–221
15. Schmalz, D., hucho, F., and Buchner, K. (1998) Nuclear import of protein kinase C occurs by a mechanism distinct from the mechanism used by proteins with classical nuclear localization signal. *J. Cell Sci.* **111**, 1823–1830
16. Haller, H., Lindschau, C., Maasch, C., Olthoff, H., Kurscheid, D., and Luft, F.C. (1998) Integrin-induced protein kinase Cα and Cε translocation to focal adhesion mediates vascular smooth muscle spreading. *Circ. Res.* **82**, 157–165
17. Vallentin, A., Prevostel, C., Fauquier, T., Bonnefont, X., and Joubert, D. (2000) Membrane targeting and cytoplasmic sequestration in the spatiotemporal localization of human protein kinase Cα. *J. Biol. Chem.* **275**, 6014–6021
18. Wallis, S., Lloyd, S., Wise, I., Ireland, G., Fleming, T.P., and Garrod, D. (2000) The a isoform of protein kinase C is involved in signaling the response of desmosomes to wounding in cul-

tured epithelial cells. *Mol. Biol. Cell* **11**, 1077–1092
19. Mochly-Rosen, D. (1995) Localization of protein kinases by anchoring proteins: A theme in signal transduction. *Science* **268**, 247–251
20. Ron, D., Chen, C., Caldwell, J., Jamieson, L., Orr, E., and Mochly-Rosen, D. (1994) Cloning of an intracellular receptor for protein kinase C: A homolog of the β subunit of G proteins. *Proc. Natl. Acad. Sci. USA* **91**, 839–843
21. Hyatt, S.L., Liao, L., Chapline, C., and Jaken, S. (1994) identification and characterization of α–protein kinase C binding proteins in normal and transformed REF52 cells. *Biochemistry* **33**, 1223–1228
22. Oka, N., Yamamoto, M., Schwencke, C., Kawabe, J., Ebina, T., Ohno, S., Couet, J., Lisanti, M.P., and Ishikawa, Y. (1997) Caveolin interaction with protein kinase C. *J. Biol. Chem.* **272**, 33416–33421
23. Kolch, W., Heidecker, G., Kochs, G., Hummel, R., Vahidi, H., Mischak, H., Finkenzeller, G., Marme, D., and Rapp, U.R. (1993) Protein kinase Cα activates Raf-1 by direct phosphorylation. *Nature* **364**, 249–252
24. Schönwasser, D.C., Marais, R.M., Marshall, C.J., and Parker, P.J. (1998) Activation of the mitogen-activated protein kinase/extracellular signal-regulated kinase pathway by conventional, novel, and atypical protein kinase C isotypes. *Mol. Cell. Biol.* **18**, 790–798
25. Sun, X. and Rotenberg, S.A. Overexpression of Protein kinase Cα in MCF-10 human breast cancer cells engenders dramatic alterations in morphology, proliferation, and motility. *Cell Growth Differ.* **10**, 343–352
26. Chang, J-H., Pratt, J.C., Sawasdikosol, S., Kapeller, R., and burakoff, S.J. (1998) The small GTP-binding protein Rho potentiates AP-trascription in T cells. *Mol. Cell. Biol.* **18**, 4986–4993
27. Taggart, M.J., Leavis, p., Feron, O., and Morgan, K.G. (2000) Inhibition of PKCα and rhoA translocation in differentiated smooth muscle by a caveolin scaffolding peptide. *Exp. Cell Res.* **258**, 72–81
28. Besson, A. and Yong, V.W. (2000) Involvement of $p21^{Waf1/Cip1}$ in protein kinase C alpha-induced cell cycle progression. *Mol. Cell. Biol.* **20**, 4580–4590
29. Frey, M.R., Saxon, M.L., Zhao, X., Rollins, A., Evans, S.S., and Black, J.D. (1997) Protein kinase C isozyme-mediated cell cycle arrest involves induction of $p21^{Waf1/Cip1}$ and $p27^{kip1}$ and hypophosphorylation of the retinoblastoma protein in intestinal epithelial cells. *J. Biol Chem.* **272**, 9429–9435
30. Frey, M.R., Clark, J.A., Leontieva, O., Uronis, J.M., Black, A.R., and Black, J.D. (2000) Protein kinase C signaling mediates a program of cell cycle withdrawal in the intestinal epithelium. *J. Cell Biol.* **151**, 763–777
31. Romanova, l.Y., Alexandrov, I.A., Nordin, R.P., Blagosklonny, M.V., and Mushinski, J.F. (1998) Cross-talk between protein kinase C-α (PKC-α) and -δ (PKC-δ). *Biochemistry* **37**, 5558–5565
32. Murakami, M., Horowitz, A., Tang, S., Ware, J.A., and Simons, M. (2002) Protein kinase C (PKC)δ regulates PKCα activity in a syndecan-4-dependnet manner. *J. Biol.Chem.*, **277**, 20367–20371
33. Deucher, A., Efimova, T., and Eckert, R.L. (2002) Calcium-dependent involucrin expression is inversely regulated by protein kinase C (PKC) α and PKCδ. *J. Biol. Chem.* **277**, 17032–17040
34. Mandil, R., Ashkenazi, E., Blass, M., Kronfeld, I., Kazimirsky, G., Rosenthal, G., Umansky, F., Lorenzo, P.S., Blumberg, P.M., and Brodie, C. (2001) Protein kinase Cα and protein kinase Cδ play opposite roles in the proliferation and apoptosis of glioma cells. *Cancer Res.* **61**, 4612–4619
35. Boudreau, R.T., Garduno, R., and Lin, T.-J. (2002) Protein phosphatase 2A and protein kinase Cα are physically associated and are involved in *Pseudomonas aeruginosa*-induced interleukin 6 production by mast cells. *J. Biol. Chem.* **277**, 5322–5329
36. Fan, H-U., Tong, C., Li, M-Y., Lian, L., Chen, D-Y., Schatten, H., and Sun, Q-Y. (2002) Translocation of the classic protein kinase C isoforms in porcine oocytes: implications of protein kinase C involvement in the regulation of nuclear activity and cortical granule exocytosis. *Exp. Cell Res.* **277**, 183–191
37. Ways, D.K., Kukoly, C.A., de Vente, J., Hooker, J.L., Bryant, W.O., Posekany, K.J., Fletcher, D.J., Cook, P.P., and Parker, P.J. (1995) MCF-7 breast cancer cells transfected with protein kinase C-α exhibit altered expression of other protein kinase C isoforms and display a more aggressive neoplastic phenotype. *J. Clin. Invest.* **95**, 1906–1915
38. Borner, C., Filipuzzi, I., Weinstein, I.B., and Imber, R. (1991) Failure of wild-type or mutant form of protein kinase C-α to transform fibroblasts. *Nature*, **353**, 78–80
39. Borner, C., Ueffling, M., Jaken, S., Parker, P.J., and Weinstein, I.B. (1995) Two closely related isoforms of protein kinase C produce reciprocal effects on the growth of rat fibroblasts. *J. Biol. Chem.* **270**, 78–86
40. Lee, J.Y., Hannum, Y.A., and Obeid, L.M. (1996) Ceramide inactivates cellular protein kinase Cα. *J. Biol. Chem.* **271**, 13169–13174
41. Chen, C-Y. and Faller, D.V. (1999) Selective inhibition of protein kinase C isozymes by Fas ligation. *J. Biol. Chem.* **274**, 15320–15328
42. Neuzil, J., Weber, T., Schröder, A., Lu M., Ostermann, G., Gellert, N., Mayne, G.C., Olejnicka, B., Nègre-Salvayre, A., Stìcha, M., Coffey, R.J., and Weber, C. (2001) Induction of cancer cell apoptosis by α-tocopheryl succinate: molecular pathways and structural requirements. *FASEB J.* **15**, 403–415
43. Jao, H.-C., Yang, R.-C., Hsu, H.-K., and Hsu, C. (2001) The decrease of PKCα is associated with hepatic apoptosis at early and late phases of polymicrobial sepsis. *Shock* **15**, 130–134
44. Whelan, R.D. and Parker, P.J. (1998) Loss of protein kinase C function induces as apoptotic response. *Oncogene* **16**, 1939–1944
45. Shen, L., Dean, N.M., and Glazer, R.I. (1999) Induction of p53-dependnet, insulin-like growth factor-binding protein-3-mediated apoptosis in glioblastoma multiforme cells by a protein kinase Cα antisense olgonucleotide. *Mol. Pharmacol.* **55**, 396–402
46. Ruvolo, P.P., Deng, X., Carr, B.K., and May, S. (1998) A functional role for mitochondrial protein kinase Cα in Bcl2 phosphorylation and suppression of apoptosis. *J. Biol. Chem.* **273**, 25436–25442
47. Okuda, H., Adachi, M., Miyazawa, M., Hinoda, Y., and Imai, K. (1999) Protein kinase Cα promotes apoptotic cell death in gastric cancer cells depending upon loss of anchorage. *Oncogene* **18**, 5604–5609
48. Shimizu, T., Cao, C-X., Shao, R-G., and Pommier, Y. (1998) Lamin B phosphorylation by protein kinase Cα and proteolysis during apoptosis in human leukemia HL60 cells. *J. Biol. Chem.* **273**, 8669–8674
49. Gschwend, J.E., Fair, W.R., and Powell, C.T. (2000) Bryostatin 1 induces prolonged activation of extracellular regulated protein kinases in and apoptosis of LNCaP human prostate cancer cells overexpressing protein kinase Cα. *Mol. Pharmacol.* **57**, 1224–1234
50. Day, M.L., Zhao, X., Vallorosi, C.J., putzi, M., Powell, T., Lin, C., and Day, K.C. (1999) E-cadherin, mediates aggregation-dependent survival of prostate and mammary epithelial cells through the retinoblastoma cell cycle control pathway. *J. Biol. Chem.* **274**, 9656–9664
51. Haslauer, M., Baltensperger, K., and Porzig, H. (1999) Erythropoietin- and stem cell factor-induced DNA synthesis in normal human erythroid progenitor cells requires activation of protein kinase Cα and is strongly inhibited by thrombin. *Blood* **94**, 114–126
52. Pierce, A., Heyworth, C.M., Nicholls, S.E., Spooncer, E., Dexter, T.M., Lord, J.M., Owen-Lynch, P.J., Wark, G., and Whetton, A.D. (1998) An activated protein kinase Ca gives a differentiation signal for hematopoietic progenitor cells and mimics macrophage colony-stimulating factor-stimulated signal events. *J. Cell Biol.* **140**, 1511–1518
53. Wagner, L.M. and Takemoto, D.J. (2001) Protein kinase Cα and γ in N/N 1003A rabbit lens epithelial cell differentiation. *Mol. Vis.* **7**, 57–62

54. Cho, Y., Klein, M.G., and Talmage, D.A. (1998) Distinct function s of protein kinase Cα and protein kinase Cβ during retinoic acid-induced differentiation of F9 cells. *Cell Growth Differ.* **9**, 147–154
55. Zhao, X., Murata, T., Ohno, S., Day, N., Song, J., Nomura, N., Nakahata, T., and Yokoyama, K.K. (2001) Protein kinase Cα plays a critical role in mannosylerythritol lipid-induced differentiation of melanoma B16 cells. *J. Biol. Chem.* **276**, 39903–39910
56. Gruber, J.R., Ohno, S., and Niles, R.M. (1992) Increased expression of protein kinase Cα play a key role in retinoic acid-induced melanoma differentiation. *J. Biol. Chem.* **267**, 13356–13360
57. Yoon, Y-M., Oh, C-D., Kang, S-S., and Chun, J-S. (2000) Protein kinase A regulates chondrogenesis of mesenchymal cells at the post-precartilage condensation stage via protein kinase C-α signaling. *J. Bone Miner. Res.* **15**, 2197–2205
58. Yu, G.,. Ahmad, S., Aquino, A., Fairchild, C.R., Trepel, J.B., Ohno, S., Suzuki, K., Tsuruo, T., Cowan, K.H., and Glazer, R.I. (1991) Transfection with protein kinase Cα confers increased multidrug resistance to MCF-7 cells expressing P-glycoprotein. *Cancer Commun.*, **3**, 181–189
59. Megidish, T. and Mazurek, N. (1989) A mutant protein kinase C that can transform fibroblasts. *Nature* **342**, 807–811
60. Vallentin, A., Lo, T.-C., and Joubert, D. (2001) A single mutation in the V3 region affects protein kinase Cα targeting and accumulation at cell-cell contacts. *Mol. Cell. Biol.* **21**, 3351–3363
61. Alvaro, V., Prevostel, C., Joubert, D., Solsberg, E., and Weinstein, B.I. (1997) Ectopic expression of a mutant form of PKCα originally found in human tumors: aberrant subcellular translocation and effects on growth control. *Oncogene* **14**, 677–685
62. Wang, X.-Y., Repasky, E., and Liu, H.-R. (1999) Antisense inhibition of protein kinase Cα reverses the transformed phenotype in human lung carcinoma cells. *Exp. Cell Res.* **250**, 253–263
63. Dean, N., Mckay, R., Miraglia, L., Howard, R., Cooper, S., Giddings, S., Nicklin, P., Meister, L., Ziel, R., Geiger, T., Muller, M., and Fabbro, D. (1996) Inhibition of growth of human tumor cell lines in nude mice by an antisense oligonucleotide inhibitor of protein kinase C-α expression. *Cancer Res.* **56**, 3499–3507
64. Radominska-Pandya, A., Chen, G., Czernik, P.J., Little, J.M., Samokyszyn, V.M., Carter, C.A., and Nowak, G. (2000) Direct interaction of all-*trans*-retinoic acid with protein kinase C (PKC). *J. Biol. Chem.* **275**, 22324–22330
65. Sil, P., Kandaswamy, V., and Sen, S. (1998) Increased protein kinase C activity in myotrophin-induced myocyte growth. *Circ. Res.* **82**, 1173–1188
66. Braz, J.C., Bueno, O.F., De Windt, L.J., and Molkentin, J.D. (2002) PKCα regulates the hypertrophic growth of cardiomyocytes through extracellular signal-regulated kinase 1/2 (ERK1/2). *J. Cell Biol.* **156**, 905–919
67. Harrington, E.O., Loffler, J., Nelson, P.R., Kent, K.C., Simons, M., and Ware J.A. (1997) Enhancement of migration by protein kinase Cα and inhibition of proliferation and cell cycle progression by protein kinase Cδ in capillary endothelial cells. *J. Biol. Chem.* **272**, 7390–7397
68. Wang, A., Nomura, M., Patan, S., and Ware J.A. (2002) Inhibition of protein kinase Cα prevents endothelial cell migration and vascular tube formation in vitro and myocardial neovascularization in vivo. *Circ. Res.* **90**, 609–616
69. St-Denis, A., Chano, F., Tremblay, P., St-Pierre, Y., and Descoteaux, A. (1998) Protein kinase C-α modulates lipopolysaccharide-induced functions in a murine macrophage cell line. *J. Biol. Chem.* **273**, 32787–32792
70. Li, S., Huang, F.L., Feng, Q., Liu, J., Fan, S.X., and McKenna, T.M. (1998) Overexpression of protein kinase Cα enhances lipopolysaccharide-induced nitric oxide formation in vascular smooth muscle cells. *J. Cell. Physiol.* **176**, 402–411
71. Wang, H.Q. and Smart, R.C. (1999) Overexpression of protein kinase C-α in the epidermis of transgenic mice results in striking alterations in phorbol ester-induced inflammation and COX-2, MIP-2 and TNF-α expression but not tumor promotion. *J. Cell Sci.* **112**, 3497–3506
72. Ohba, M., Ishino, K., Kashiwagi, M., Kawabe, S., Chida, K., Huh, N.H., and Kuroki, T. (1998) Induction of differentiation in normal human keratinocytes by adenovirus-mediated introduction of the η and δ isoforms of protein kinase C. *Mol. Cell. Biol.* **18**, 5199–5207
73. Ozawa, K., Szallasi, Z., Kazanietz, M.G., Blumberg, P.M., Mischak, H., Mushinski, J.F., and Beaven, M.A. (1993) Ca^{2+}-dependent and Ca^{2+}-independent isozymes of protein kinase C mediate exocytosis in antigen-stimulated rat basophilic RBL-2H3 cells. *J. Biol. Chem.* **268**, 1749–1756
74. Chang, E.-Y., Szallasi, Z., Acs, P., Raizada, V., Wolfe, P.C., Fewtrell, C., Blumberg, P.M., and Rivera, J. (1997) Functional effects of overexpression of protein kinase-Cα, -β, -δ, -ε, and -η in the mast cell line RBL-2H3. *J. Immunol.* **159**, 2624–2632
75. Han, Y., Meng, T., Murry, N.R., Fields, A.P., and Brasier, A.R. (1999) Interleukin-1-induced nuclear factor–κ-B-IκBα autoregulatory feedback loop in hepatocytes. *J. Biol. Chem.* **274**, 939–947